Oxidation of Biological Substrates by Chromium(VI). Part 1. Mechanism of the Oxidation of L-Ascorbic Acid in Aqueous Solution

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The kinetics of oxidation of L-ascorbic acid (H_2A) by potassium chromate has been studied under aerobic and anaerobic conditions over the range $0.002 \leqslant [A]_T \leqslant 0.040$ mol dm^{-3}, $3.50 \leqslant pH \leqslant 8.70$, $17.8 \leqslant T \leqslant 35.1$ °C, $0.06 \leqslant I \leqslant 0.50$ mol dm^{-3} ($NaClO_4$) and $0.05 \leqslant [O_2] \leqslant 0.12$ mmol dm^{-3}. The experimental rate constants obtained in the presence of oxygen are about 10 times smaller than those obtained in its absence. A mechanism involving the formation of a chromium(VI)–ascorbate–oxygen intermediate is discussed in an attempt to explain this. The disappearance of chromium(VI) under aerobic conditions follows the rate law (i) where $k_f = (k_1K_{a1}[H^+] + k_2K_{a1}K_c + k_3[H^+]^2)/(K_{a1} + [H^+])(K_c + [H^+])$.

$$-d[Cr^{VI}]/dt = k_f[A]_T[Cr^{VI}]_T \quad \text{(i)}$$

At 25 °C and $I = 0.50$ mol dm^{-3} ($NaClO_4$), $k_1 = 59.4 \pm 0.5$, $k_2 = 0.10 \pm 0.02$ and $k_3 = 115 \pm 3$ dm^3 mol^{-1} s^{-1}.

L-Ascorbic acid (H_2A) is very widely known and used for its reducing properties.[1–10] Among the many reactions studied are those involving its efficient reduction of many transition-metal ions and complexes by outer- and inner-sphere mechanisms.[11–13]

It has also been shown that L-ascorbic acid is oxidized by the potentially carcinogenic chromium(VI) ion at the body's physiological pH of 7.40, as well as at lower pH.[14–16] The use of chromate as an oxidant is now discouraged as a result of its link to the formation of skin cancers.[17] This carcinogenicity is thought to be related to the oxidation of various cellular constituents. Ascorbic acid, being a constituent of the cell, and a good reductant, may therefore function as an antichrome agent *in vivo* against chromate poisoning.[14,15,17]

Connett and Wetterhahn[14,15,17] observed that various intercellular metabolites, including ascorbic acid, are capable of reducing chromate under physiological conditions. However, their reports were somewhat limited in kinetic and mechanistic details. We have therefore extended the study to involve a wider range of pH, ascorbic acid concentration and ionic strength. Most interesting, however, is our observation that molecular oxygen is involved in the reaction. This paper reports the results of a comprehensive study of the oxidation of L-ascorbic acid by the chromium(VI) ion under aerobic and anaerobic conditions.

Experimental

Materials.—The chemicals used were either analytical or of the desired reagent grade (BDH), used as received. The purity of L-ascorbic acid was determined iodometrically.[18] The concentration of hexaaquachromium(III) perchlorate was determined by complexometric[19] titration and spectrophotometry.[20] The deionized water used in all experimental work was obtained by passing distilled water through a Milli-Q Reagent grade water system (Millipore Corporation, Bedford, MA). Nitrogen was supplied by Jamaica Oxygen and Acetylene Limited. Microanalysis was done by Butterworth Laboratories, UK.

Preparation of Chromium(III)–Ascorbate Complex.—The complex was prepared from K_2CrO_4 (0.088 g) in water (25 cm^3) and L-ascorbic acid (0.34 g). The reaction was allowed to proceed for 0.5 h at about 29 °C after adjustment to pH 7 with KOH. The green solution was concentrated by heating at 40 °C. The viscous liquid formed was cooled in ice, methanol added, and further cooled in ice. The green product was filtered off, washed with cold methanol and placed in a desiccator. Yield 0.33 g. UV/VIS: λ_{max}/nm 590 and 410 (ε, 38 and 61 dm^3 mol^{-1} cm^{-1}) {Found: C, 25.5; H, 2.9; Cr, 10.5; K, 11.7. $K[Cr(C_6H_6O_6)_2(OH)_2]\cdot 0.5KOH$ requires C, 28.7; H, 2.9; Cr, 10.4; K, 11.7%}.

Stoichiometry.—The redox stoichiometry [ascorbate: Cr^{VI}] was determined by measuring the absorbance at 550 nm as a function of the ratio $[Cr^{VI}]$:[ascorbate], while varying [ascorbate].

Ion-exchange Properties.—Ion-exchange resins, Na^+ and Cl^- forms (Dowex-50W, 100–200 mesh), were used. The solutions from the mixtures were chromatographed on both resins. Green and brown bands were eluted from the Na^+ resin using water. Most of the green product was removed with 0.50 mol dm^{-3} NaCl solution from the Cl^- resin. The brown band was removed with 4 mol dm^{-3} NaCl from the Cl^- resin.

Kinetics.—Kinetic studies were conducted by monitoring the absorbance of the chromate ion at 370 nm *vs.* time under pseudo-first-order conditions of excess of ascorbic acid. Constant ionic strength was maintained at 0.50 mol dm^{-3} ($NaClO_4$). Phosphate–citrate and Tris [tris(hydroxymethyl)-methylamine]–HCl were used to maintain the lower and higher pH regions respectively. All pH measurements were done using an Orion model 701 pH meter, fitted with a Cole Parmer combination electrode. The faster reactions were studied using a Hi-Tech scientific model SF-51 stopped-flow unit connected to a SU-40 UV/VIS spectrophotometer. Constant temperature was maintained with a Haake GH constant-temperature water-bath fitted with a Haake D8 circulating pump.

The slower reactions were studied using the spectrophotometric method. Solutions for anaerobic study were flushed with oxygen-free nitrogen while being equilibrated. They were protected by rubber serum caps and handled by standard syringe techniques. The oxygen concentration in the

kinetic solutions was measured using a YSI Scientific Water Quality instrument, fitted with a YSI model 5739 oxygen electrode.

Pseudo-first-order rate constants for the slower reactions were determined using a STATGRAPHICS computer program[21] in the non-linear mode. All plots of $\ln(A_t - A_\infty)$ *vs.* time were linear for at least three half-lives.

Spectral Measurements.—The UV/VIS spectra were recorded using either a Varian-Cary 219 or a Hewlett-Packard 8452A diode-array spectrophotometer. These were connected to thermostatted water-baths maintained to ±0.1 °C.

Results

The addition of ascorbate to a solution of chromium(VI) at pH 7.4 resulted in the formation of a green complex. During repetitive scanning of the reaction the chromate peak at 370 nm gradually disappeared, while a peak at 550–600 nm indicative of chromium(III) species was formed (Figs. 1 and 2). No clear isosbestic point was seen in the repetitive scanning (Fig. 1), hence intermediates were probably formed during the reaction. A spectrum of the green product was similar to that of a solution of hexaaquachromium(III) in ascorbate under the same conditions.

The green product slowly turned brown in air. This was due to the formation of products involving dehydroascorbic acid[22] (Fig. 2) and chromium(III), as was confirmed by matching the spectrum of this solution with that of a synthetic mixture of dehydroascorbic acid and hexaaquachromium(III) which also appeared brown after ≈30 min. In the absence of oxygen the green colour persisted.

Characterization of the Major Product.—Additional evidence for the formation of chromium(III) was provided by ethylenediaminetetraacetate (edta) titration.[19] Microanalytical results indicated that the green complex contained about 2 mol of ascorbate to each chromium(III) ion. Some potassium hydroxide present as impurity could not easily be removed.

Stoichiometry of the Reaction.—The stoichiometric data in Table 1 are consistent with the reaction summarized in equation (1) [H_2A = ascorbic acid, A′ = dehydroascorbic acid]. This result corroborates well with that determined at a lower pH by an entirely different method.[16]

$$3HA^- + 2[HCrO_4^-]^- + 3H_2O \longrightarrow 2Cr^{3+} + 3A' + 11OH^- \quad (1)$$

Ascorbic Acid and pH *Dependence of the Reaction.*—Kinetic runs were carried out at 25.0 °C with the ascorbic acid concentration ranging from 0.002 to 0.040 mol dm^{-3} over the range pH 4.60–7.40, at an ionic strength of 0.50 mol dm^{-3} in $NaClO_4$. The pseudo-first-order rate constants increase with increasing ascorbate concentration, and decrease as the pH is increased at a fixed ascorbate concentration (Table 2).

Plots of k_{obs} *vs.* $[ascorbate]_T$ were linear with small intercepts (Fig. 3) which decrease with increasing pH. These results indicate that the reaction is first order with respect to ascorbate

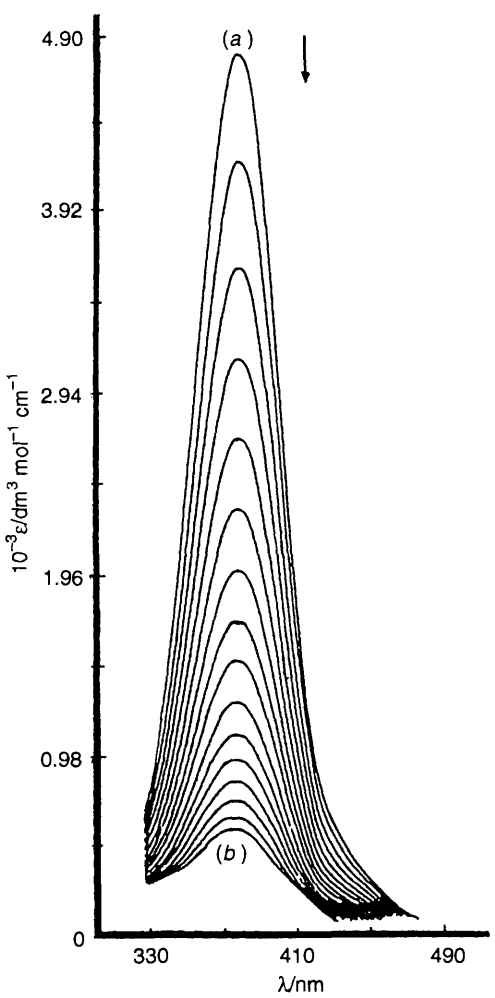


Fig. 1 Spectral changes for the oxidation of L-ascorbic acid by chromium(VI) at $[CrO_4^{2-}]_T = 1.85 \times 10^{-3}$ mol dm^{-3}, $[ascorbate]_T = 3.3 \times 10^{-3}$ mol dm^{-3}, pH 7.3, cycle time = 1 min, 25 °C: (*a*) CrO_4^{2-}, (*b*) 15 min after start of reaction

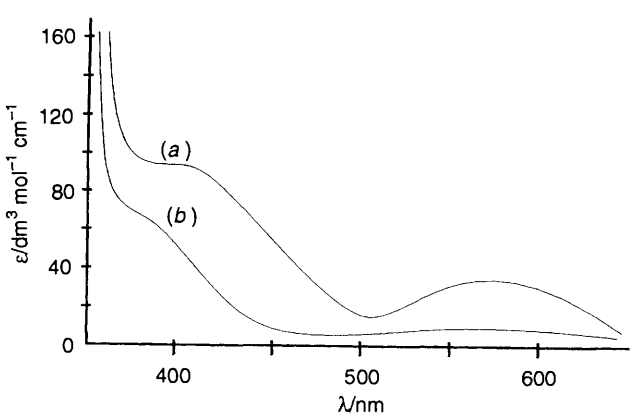


Fig. 2 Spectra of the products of reaction between chromium(VI) and L-ascorbic acid at pH 7: (*a*) green product, (*b*) brown product

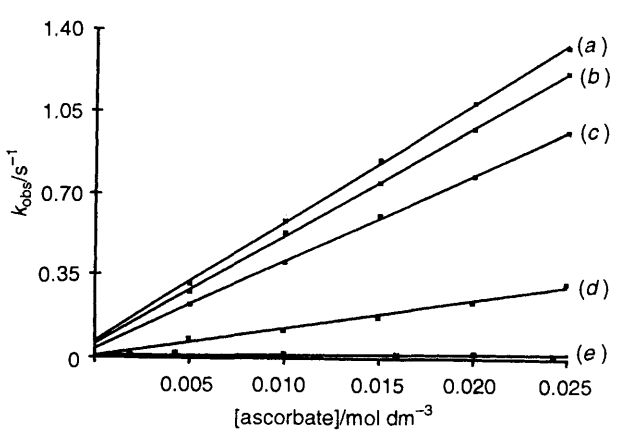


Fig. 3 Plot of k_{obs} *vs.* $[ascorbate]_T$ for the oxidation of L-ascorbic acid by chromium(VI) at different pH, 25 °C, I = 0.50 mol dm^{-3}. pH: 4.6 (*a*), 4.84 (*b*), 5.47 (*c*), 6.45 (*d*) and 7.40 (*e*)

Table 1 Stoichiometry of the reaction between L-ascorbic acid and chromium(VI) at 25 °C, pH 7.40 (Tris–HCl buffer), I = 0.50 mol dm^{-3} ($NaClO_4$); $[CrO_4^{2-}]_T$ = 7.0 × 10^{-3} mol dm^{-3}, λ = 550 nm, cell pathlength = 1 cm

$[H_2A]/[CrO_4^{2-}]$	Absorbance	$[H_2A]/[CrO_4^{2-}]$	Absorbance
0	0	1.75	0.440
0.25	0.120	2.00	0.435
0.50	0.210	2.50	0.440
0.75	0.275	5.00	0.440
1.00	0.310	7.00	0.430
1.50	0.430*	10.0	0.425

* Break point occurs at $[H_2A]/[CrO_4^{2-}]$ = 1.5.

Table 2 Pseudo-first-order rate constants for the reaction between L-ascorbic acid and chromium(VI). Effect of pH variation (phosphate–citrate buffer) at I = 0.50 mol dm^{-3} ($NaClO_4$), $[CrO_4^{2-}]_T$ = 2.0 × 10^{-4} mol dm^{-3}; [ascorbate] = 5.0 × 10^{-3} mol dm^{-3}, 25.0 °C

pH	k_{obs}/s^{-1} (17.8 °C)	pH	k_{obs}/s^{-1} (21 °C)	pH	k_{obs}/s^{-1} (25 °C)	pH	k_{obs}/s^{-1} (30.1 °C)	pH	k_{obs}/s^{-1} (35.1 °C)
3.93	0.24	3.69	0.35	3.75	0.47	3.79	0.60	3.53	0.82
4.24	0.22	3.81	0.34	3.87	0.44	3.83	0.59	3.55	0.74
4.40	0.20	4.02	0.31	4.00	0.44	3.96	0.57	3.70	0.74
5.42	0.13	4.14	0.31	4.11	0.41	4.12	0.54	4.07	0.71
5.72	0.11	4.24	0.30	4.28	0.39	4.22	0.52	4.20	0.67
5.80	0.10	4.38	0.28	4.44	0.37	4.28	0.52	4.44	0.63
6.01	0.09	4.77	0.26	4.62	0.35	4.30	0.51	4.72	0.59
6.10	0.08	5.14	0.20	4.81	0.33	4.44	0.48	4.77	0.55
6.23	0.07	5.66	0.14	5.07	0.29	4.51	0.47	4.94	0.53
6.41	0.06	6.08	0.11	5.13	0.27	4.62	0.45	5.32	0.48
6.60	0.03	6.20	0.09	5.39	0.25	4.68	0.43	5.33	0.39
7.20	5.30[a,b]	6.32	0.08	5.36	0.22	4.92	0.40	5.48	0.35
7.35	3.90[a,b]	6.40	0.07	5.57	0.19	5.01	0.42	5.60	0.44
7.39	3.59[a,b]	6.46	0.06	5.72	0.17	5.13	0.41	5.73	0.38
7.49	2.83[a,b]	6.58	0.05	6.34	0.10	5.16	0.35	5.74	0.33
7.51	2.73[a,b]	7.14	8.24[a,b]	6.57	0.07	5.29	0.34	5.79	0.32
7.75	1.37[a,b]	7.29	6.40[a,b]	7.09	11.9[a]	5.33	0.32	5.89	0.28
7.85	1.07[a,b]	7.49	4.17[a,b]	7.41	6.57[a]	5.35	0.37	5.97	0.28
7.90	1.10[a,b]	7.50	4.37[a,b]	7.45	5.57[a]	5.48	0.31	6.09	0.24
8.56	0.72[a,b]	7.53	3.90[a,b]	7.53	5.03[a]	5.57	0.28	6.20	0.24
8.77	0.43[a,b]	7.61	3.24[a,b]	7.60	4.98[a]	5.64	0.32	6.21	0.22
		7.64	3.09[a,b]	7.66	3.91[a]	5.93	0.22	6.24	0.20
		7.81	1.85[a,b]	7.75	3.29[a]	6.07	0.26	6.36	0.18
		7.89	2.22[a,b]	7.76	3.66[a]	6.10	0.18	6.45	0.19
		8.03	1.60[a,b]	7.85	3.56[a]	6.22	0.20	6.48	0.16
		8.35	1.13[a,b]	8.08	1.87[a]	6.32	0.15	6.57	0.15
		8.75	0.84[a,b]	8.37	1.37[a]	6.51	0.12		
				8.55	0.93[a]				

[a] $10^3 k_{obs}$/s^{-1}. [b] $[CrO_4^{2-}]_T$ = 3.7 × 10^{-4} mol dm^{-3}; $[ascorbate]_T$ = 1.0 × 10^{-2} mol dm^{-3}.

Table 3 Kinetic parameters obtained from plots of k_{obs} *vs.* $[ascorbate]_T$ for the reaction between L-ascorbic acid and chromium(VI) at different pH, 25.0 °C, I = 0.50 mol dm^{-3}

pH	k_f/dm^3 mol^{-1} s^{-1}	k_r/s^{-1}	$10^{-2} k_f k_r^{-1}$/dm^3 mol^{-1}
4.60	50.9 ± 0.9	(6.87 ± 1.42) × 10^{-2}	7.40 ± 1.53
4.84	46.7 ± 0.7	(5.75 ± 1.22) × 10^{-2}	9.06 ± 1.93
5.47	37.3 ± 0.5	(3.72 ± 0.87) × 10^{-2}	10.02 ± 2.35
6.55	13.0 ± 0.1	—	—
7.40	0.56 ± 0.02	(5.83 ± 3.57) × 10^{-4}	9.63 ± 5.91
7.40*	0.60 ± 0.02	—	—

* Ref. 16.

concentration and also that there is a second step independent of ascorbate concentration making some minor contribution, especially at lower pH. The rate-determining step of the reaction can therefore be of the form (2). The product of this reaction is

$$H_2A + [HCrO_4]^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{products} \qquad (2)$$

a chromate–ascorbate ester intermediate. The resulting rate expression is as in equation (3). Values for k_f and k_r were

$$k_{obs} = k_f[ascorbate]_T + k_r \qquad (3)$$

evaluated directly from the slopes and intercepts respectively of the plots of k_{obs} *versus* [ascorbate] and are given in Table 3. All values for k_f are much larger than those for k_r, indicating that this is the major route accounting for the disappearance of the chromium(VI). Estimates of the equilibrium constant, K_e, for the reaction were obtained from the ratio k_f/k_r. These were found to vary from 7.4 × 10^2 to 1.0 × 10^3 dm^3 mol^{-1} at 25 °C depending on the pH.

Effect of Ionic Strength.—A change in ionic strength from 0.06 to 0.20 mol dm^{-3} at pH 4 produced an increase of 0.03 s^{-1} in the experimental rate constants, while at pH 7.4 a decrease in ionic strength from 0.50 to 0.29 mol dm^{-3} gave an increase of 2.17 × 10^{-3} s^{-1}.

Effect of Anaerobic Conditions.—The oxygen concentration was varied from 0.05 to 0.12 mmol dm^{-3}. Under anaerobic conditions L-ascorbic acid rapidly reduces chromium(VI) at

Table 4 First-order rate constants for the reaction between L-ascorbic acid and chromium(VI): effect of oxygen concentration at 25.2 °C, pH 7.70 (Tris–HCl buffer), I = 0.50 mol dm^{-3}, [ascorbate]$_T$ = 1.0 × 10^{-3} mol dm^{-3}, [CrO_4^{2-}]$_T$ = 2.0 × 10^{-4} mol dm^{-3}

10^4[O_2]/mol dm^{-3}	10^2k_{obs}/s^{-1}	10^4[O_2]/mol dm^{-3}	10^2k_{obs}/s^{-1}
0.50	3.14	0.80	1.94
0.59	2.80	0.86	1.71
0.61	2.79	0.88	1.67
0.62	2.70	0.89	1.44
0.66	2.58	0.91	1.31
0.74	2.22	0.94	1.31
0.75	2.17	1.17	0.21
0.77	2.08	1.19	0.23

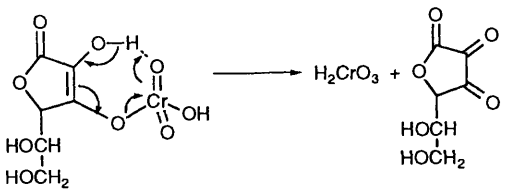
a rate which is about ten times faster than the rates of reactions carried out in oxygen. The rate constants increase as oxygen concentration decreases (Table 4). The rate constants also increase with decreasing pH, similar to the reaction studied under aerobic conditions: k_{obs} 2.95, 2.60, 2.16, 1.32, 0.61 and 0.31 s^{-1} at pH 7.70, 7.78, 7.85, 8.09, 8.39 and 8.77 respectively, at [O_2] = 0.06 mmol dm^{-3}.

Discussion

The reduction of chromate by ascorbate occurs *via* the formation of a chromate–ascorbate ester intermediate in the rate-determining step. This intermediate undergoes rapid electron transfers, *via* an inner-sphere type mechanism, to produce chromium(III) and dehydroascorbate as final products.

The estimates of the equilibrium constant, K_e, for the chromate–ascorbate ester formation (740–1000 dm^3 mol^{-1}), obtained in our study are similar to that reported (K = 250 dm^3 mol^{-1}) for the formation of the oxygen-bonded chromate–isopropyl alcohol ester.[23a]

The formation of chromate–ester intermediates, by these organic substrates,[23–28] apparently provides a low-energy pathway for the transfer of electrons[29] *via* an inner-sphere mechanism. The ascorbate–chromate intermediate formed may be of the form shown on the left of equation (4) and can undergo



(4)

electron transfer as indicated. Its formation is favoured at lower pH, as the undissociated ascorbic acid, H_2A, can lose a proton to an OH of [$HCrO_4$]$^-$ thereby increasing the lability of a Cr–O bond. This ensures the easy loss of a molecule of water.[14,30] This proposed intermediate can decompose *via* a second route without the transfer of electrons. It may revert to the original reactants, and it is this pathway which is described by the rate constant k_r in equation (2).

The pH dependence of the rate constants suggests that [$HCrO_4$]$^-$, H_2A and HA^- are the reactive species in the rate-determining step producing the ester. The very small concentration of chromium(VI) was such that the amount of the dimeric product, [Cr_2O_7]$^{2-}$, was negligible at all pH values.[31] Below pH 5, H_2A is the dominant form of the ascorbic acid, whereas above this pH the monoanionic form is predominant. Equation (2) can therefore be represented more completely in terms of these reactive species as shown in Scheme 1. The

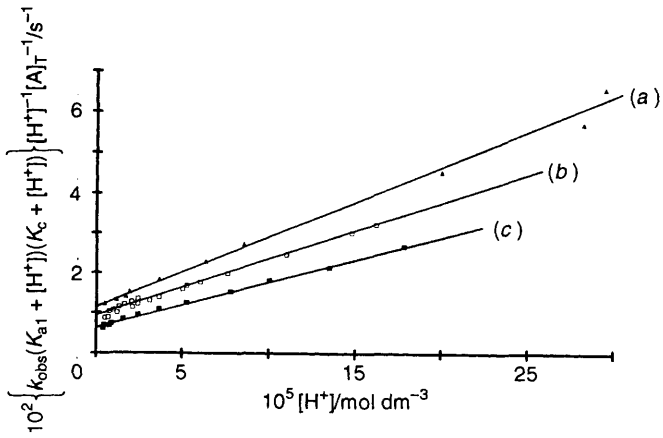


Fig. 4 Plot of $[k_{obs}(K_{a1} + [H^+])(K_c + [H^+])]/[H^+][A]_T$ *vs.* [H^+] for the oxidation of L-ascorbic acid by chromium(VI) at different temperatures, I = 0.50 mol dm^{-3}: (*a*) 35.1, (*b*) 30.1 and (*c*) 25.0 °C

$$H_2A \overset{K_{a1}}{\rightleftharpoons} HA^- + H^+ \quad (5)$$

$$HA^- \overset{K_{a2}}{\rightleftharpoons} A^{2-} + H^+ \quad (6)$$

$$[HCrO_4]^- \overset{K_c}{\rightleftharpoons} [CrO_4]^{2-} + H^+ \quad (7)$$

$$HA^- + [HCrO_4]^- \xrightarrow{k_1} \text{products} \quad (8)$$

$$HA^- + [CrO_4]^{2-} \xrightarrow{k_2} \text{products} \quad (9)$$

$$H_2A + [HCrO_4]^- \xrightarrow{k_3} \text{products} \quad (10)$$

$$H_2A + [CrO_4]^{2-} \xrightarrow{k_4} \text{products} \quad (11)$$

Scheme 1 K_{a1} = 1.05 × 10^{-4} dm^3 mol^{-1}, K_{a2} = 2.5 × 10^{-12} and K_c = 1.05 × 10^{-6} mol dm^{-3}

overall rate law consistent with this scheme is given in equation (12). After making the relevant substitutions for the

$$\text{rate} = (k_1[HCrO_4^-] + k_2[CrO_4^{2-}])[HA^-] + (k_3[HCrO_4^-] + k_4[CrO_4^{2-}])[H_2A] \quad (12)$$

concentrations of each species, equation (13) is obtained. Comparing equation (3) with (13), it can be seen that k_f is given

$$k_{obs} = \frac{(k_1K_{a1}[H^+] + k_2K_{1a}K_c + k_3[H^+]^2)}{(K_{a1} + [H^+])(K_c + [H^+])}[A]_T + k_r \quad (13)$$

by (14). Since k_r represents only a minor reaction it can be

$$k_f = \frac{(k_1K_{a1}[H^+] + k_2K_{a1}K_c + k_3[H^+]^2)}{(K_{a1} + [H^+])(K_c + [H^+])} \quad (14)$$

excluded from further analysis. It was found that the best fits for the rate constants were obtained by considering the k_1 and k_3 pathways as being the significant ones below pH 5 while k_1 and k_2 were taken as contributing most to the reaction above pH 5. The k_1 and k_4 paths are mechanistically identical, but indistinguishable. Separate non-linear regression analyses were done at the lower pH values to obtain values for k_1 and k_3 (Fig. 4), and at the higher pH values to obtain values for k_1 and k_2 using the appropriate rate expressions in a STATGRAPHICS computer program. Values for k_1 obtained from analyses of the two pH regions were found to be in good agreement. The rate constants and the calculated

Table 5 Rate constants and activation parameters for the reaction between L-ascorbic acid and chromium(VI)

T/°C	$10^{-1}k_1$/dm^3 mol^{-1} s^{-1}	k_2/dm^3 mol^{-1} s^{-1}	$10^{-1}k_3$/dm^3 mol^{-1} s^{-1}
17.3	3.52 ± 0.02	0.03 ± 0.01*	5.86 ± 0.40
21.1	4.77 ± 0.03	0.05 ± 0.01	8.30 ± 0.13
25.0	5.94 ± 0.05	0.10 ± 0.02	11.5 ± 0.3
30.1	8.99 ± 0.18		14.7 ± 0.3
35.1	10.6 ± 0.2		17.2 ± 0.3

$\Delta S_1^{\ddagger} = -62.4 \pm 11.2, \Delta S_2^{\ddagger} = 69 \pm 133, \Delta S_3^{\ddagger} = -63.9 \pm 15.2$ J K^{-1} mol^{-1}, $\Delta H_1^{\ddagger} = 44.2 \pm 3.0$, $\Delta H_2^{\ddagger} = 105 \pm 32$, $\Delta H_3^{\ddagger} = 42.4 \pm 4.1$ kJ mol^{-1}.

* Estimates derived from data above pH 7.

activation parameters[31] are listed in Table 5. Values for k_1 and k_3 can also be evaluated from equation (14) using data from the ascorbate dependence of the reaction. From a linear regression analysis a reasonably good fit was obtained especially for k_f values at lower pH, in support of the proposed mechanism in Scheme 1.

The pathway represented by k_3 has the largest rate constant indicating that formation of the chromate–ascorbate intermediate is promoted by the presence of the extra proton, in accord with earlier results.[14,22a,30,32–37] The reaction between $[CrO_4]^{2-}$ and HA^- at higher pH is quite sluggish as expected, since in this case the leaving group is not water but the less preferred OH^-.[14]

The magnitude of the entropy of activation for the k_1 and k_3 pathways suggests some degree of organization in the transition states associated with these pathways. This is consistent with the associative nature of the mechanism. The present activation parameters conform to those obtained by Banas[16] for the oxidation of ascorbic acid by chromic acid, where $\Delta S^{\ddagger} = -69.5 \pm 6$ J K^{-1} mol^{-1} and $\Delta H^{\ddagger} = 28.5 \pm 1.8$ kJ mol^{-1}.

In earlier studies, Connett and Wetterhahn[14] proposed a mechanism for the ascorbate–chromium(VI) reaction at neutral pH, where a chromate ester intermediate was formed and was capable of undergoing an internal electron transfer [equations (15) and (16)]. Assuming steady-state conditions for this

$$HA^- + [HCrO_4]^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A\text{–}Cr^{VI} + H_2O \quad (15)$$

$$A\text{–}Cr^{VI} \xrightarrow{k_2} Cr^{IV} \quad (16)$$

intermediate, and that the reverse reaction is small compared to the redox reaction (*i.e.* $k_2 \geqslant k_{-1}$), equation (17) can be written

$$-d[Cr^{VI}]/dt = k_1[HA^-][Cr^{VI}] \quad (17)$$

where k_1 is the rate constant for the formation of the ester intermediate which equates to k_f in this work. These authors reported a value of 0.60 ± 0.02 dm^3 mol^{-1} s^{-1} for k_1 which seems quite reasonable, *cf.* our k_f value of 0.56 ± 0.02 dm^3 mol^{-1} s^{-1} for the reduction of $[HCrO_4]^-$ by HA^- at pH 7.4. This study conclusively shows that k_r (k_{-1} from the literature) is insignificant.

Based on the species reacting in the rate-limiting step, an increase in ionic strength at low pH should produce no significant changes in the rate constants. This was in fact observed. At higher pH where an increase in the ionic strength should cause an increase in rate constants, there was a 30% decrease! The reason for this is not quite understood.

The three-unit change in oxidation number for the chromium(VI) reduction contrasts sharply with the single net electron change for most transition-metal complexes. Consequently, it is believed that these reactions occur in a sequence of steps, involving intermediate valence states of chromium, namely Cr^V and Cr^{IV}.[24–28,33–42] More recently, some experimental evidence was presented for the first time

Table 6 Pseudo-first-order rate constants for the reaction between L-ascorbic acid and chromium(VI). Effect of Mn^{2+} and I^- on rate of reaction at $I = 0.50$ mol dm^{-3} ($NaClO_4$), 25.0 °C, pH 2.33 (phosphate–citrate buffer), $[CrO_4^{2-}]_T = 2.0 \times 10^{-4}$ mol dm^{-3}, $[\text{ascorbate}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}

$10^3[Mn^{2+}]$/mol dm^{-3}	k_{obs}/s^{-1}	$10^3[I^-]$/mol dm^{-3}	k_{obs}/s^{-1}
1.5	0.621	1.5	0.625
3.0	0.623	3.0	0.616
3.0[b]	0.061	3.0[b]	0.126
4.5	0.607	6.0	1.183
1.5[a]	5.81×10^{-3}	*c*	0.645[b]
3.0[a]	9.45×10^{-3}	3.0[b]	0[c]
1.5[a,b]	0	3.0	6.31×10^{-3}
3.0[a,b]	0	1.5	6.46×10^{-3}

[a] pH 7.40 (Tris–HCl buffer). [b] No Mn^{2+} or I^- added. [c] No ascorbate added.

Table 7 Stoichiometry of the reaction between L-ascorbic acid and chromium(VI): effect of Mn^{2+} at 25.0 °C, pH 7.40 (Tris–HCl buffer), $I = 0.50$ mol dm^{-3} ($NaClO_4$), $[CrO_4^{2-}]_T = 2.0 \times 10^{-4}$ mol dm^{-3}, $[Mn^{2+}] = 3.0 \times 10^{-3}$ mol dm^{-3}, $\lambda = 370$ nm, cell path length = 1 cm

$[H_2A]/[CrO_4^{2-}]$	Absorbance	$[H_2A]/[CrO_4^{2-}]$	Absorbance
0.25	0.714	1.75	0.025
0.50	0.536	2.00	0.036
0.75	0.377	2.50	0.042
1.00	0.222	5.00	0.046
1.25	0.114	7.50	0.060
1.50	0.037*	10.0	0.060

* Break point in data occurs at $[H_2A]/[CrO_4^{2-}] = 1.50$.

implicating the involvement of a chromium(II) intermediate during the reduction of chromium(VI) by formaldehyde.[43]

Goodgame and Joy[44] utilized ESR spectroscopy to study the ascorbate–chromium(VI) reaction, and, based on their findings, further support was obtained for the production of some chromium(V) species as well as ascorbate radicals. Recent theoretical studies of the general mechanism of oxidation of ascorbic acid, using molecular orbital calculations, invoke formation of the ascorbate radical as the initial product in neutral or basic solutions.[45,46] It is thought that the reduction of chromium(V) to the chromium(IV) state is the slowest, owing to reorganization involved in the expansion of the co-ordination shell from tetrahedral to octahedral required for the chromium(IV) state.

Manganese(II) and I^- ions have been used to trap the chromium-(IV) and -(V) intermediates respectively [equations (18) and (19)].[47–49] These are induction reactions and can

$$Mn^{II} + Cr^{IV} \longrightarrow Mn^{III} + Cr^{III} \quad (18)$$

$$2I^- + Cr^V \longrightarrow I_2 + Cr^{III} \quad (19)$$

influence directly the overall mechanism or stoichiometry.[39] In this work however, the use of these 'scavengers' did not have any meaningful effect on the experimental rate constants or the redox stoichiometry (Tables 6 and 7).

The work of Gould and co-workers[10,50,51] involving the oxidation of L-ascorbic acid by chromium-(IV) and -(V) complexes highlights the rapid nature of the electron-transfer reactions. The rate of reduction of the chromium(V) 2-ethyl-2-hydroxybutyrate chelate to the chromium(IV) species is, for example, of the order of 2×10^2 dm^3 mol^{-1} s^{-1}, while the internal electron transfer from chromium(IV) to chromium(III) ascorbate chelate is 2×10^3 s^{-1}. These rate constants for the one-electron reductions are in accord with the assumption that the formation of the ester intermediate is rate determining.

Experimentally, it was observed that a chromium(III)–

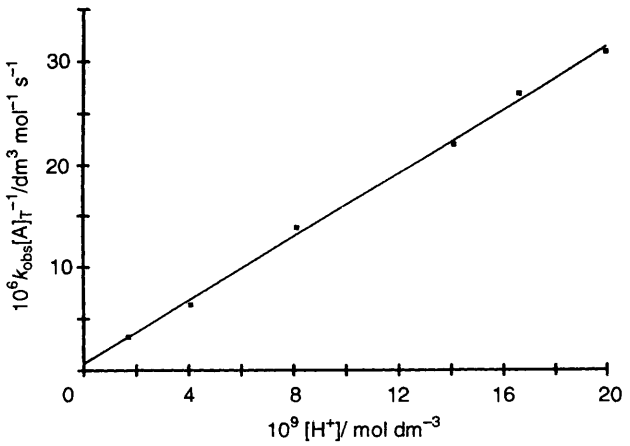


Fig. 5 Plot of $k_{obs}/[A]_T$ *vs.* $[H^+]$ for the oxidation of L-ascorbic acid by chromium(VI) under anaerobic conditions at 25 °C, I = 0.50 mol dm^{-3}

ascorbate product was formed within 5 min of the addition of L-ascorbic acid to chromium(VI). The fact that, even in excess, ascorbate takes a much longer time to form a similar product with hexaaquachromium(III), under identical experimental conditions, implies that in reacting with chromium(VI) the ascorbate does not co-ordinate to chromium(III), but to labile chromium-(V) or -(IV) states,[41] which are then reduced to chromium(III). This is summarized in Scheme 2. Further

$$H_2A \overset{K_{a1}}{\rightleftharpoons} HA^- + H^+ \quad (5)$$

$$HA^- \overset{K_{a2}}{\rightleftharpoons} A^{2-} + H^+ \quad (6)$$

$$[HCrO_4]^- \overset{K_c}{\rightleftharpoons} [CrO_4]^{2-} + H^+ \quad (7)$$

$$HA^- + Cr^{VI} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} HA\text{–}Cr^{VI} \quad (20)$$

$$HA\text{–}Cr^{VI} \xrightarrow[\text{fast}]{k_2} Cr^V + HA^\bullet \quad (21)$$

$$Cr^V + HA^\bullet \xrightarrow[\text{fast}]{k_3} Cr^{IV} + H^+ + A' \quad (22)$$

$$Cr^{VI} + HA^\bullet \xrightarrow[\text{fast}]{k_4} Cr^V + H + A' \quad (23)$$

$$Cr^V + HA^- \xrightarrow[\text{fast}]{k_5} Cr^{IV} + HA^\bullet \quad (24)$$

$$Cr^{IV} + HA^- \xrightarrow[\text{fast}]{k_6} Cr^{III} + HA^\bullet \quad (25)$$

$$Cr^{IV} + HA^\bullet \xrightarrow[\text{fast}]{k_7} Cr^{III} + H^+ + A' \quad (26)$$

Scheme 2 Cr^{VI} is $[HCrO_4]^-$ or $[CrO_4]^{2-}$, and $HA^\bullet$ is ascorbate radical, A′ = dehydroascorbic acid

support for the co-ordination to labile chromium intermediates comes from a report on the reaction of chromium(VI) with edta.[52]

Kinetics under Anaerobic Conditions.—From the pH dependence of the reaction it can be assumed that the reactive forms of chromate and ascorbate are the same as when the reaction is performed under aerobic conditions. From Scheme 1, HA^- and $HCrO_4^-$ are reactive at pH 7.70 [equations (6)–(9)], hence equation (27) is applicable. Under the experimental conditions

$$k_{obs} = \frac{k_1[H^+] + k_2K_c}{(K_{a1} + [H^+])(K_c + [H^+])} K_{a1}[A_T] \quad (27)$$

used, $K_{a1} \gg [H^+]$ and $K_c \gg [H^+]$. Equation (27) can be reduced to the form (28). A plot of the left-hand side of equation

$$\frac{k_{obs}}{[A_T]} = \frac{k_1[H^+]}{K_c} + k_2 \quad (28)$$

(28) against $[H^+]$ produced a straight line with a small intercept (Fig. 5). The value of k_1 was calculated as 1542 ± 40 dm^3 mol^{-1} s^{-1} compared to 59.4 ± 0.5 dm^3 mol^{-1} s^{-1} obtained under aerobic conditions. The magnitude of the intercept indicates that the k_2 path is of little significance.

A number of possibilities were considered in an attempt to explain how molecular oxygen retards the rate of oxidation of L-ascorbic acid by chromium(VI). These included the spontaneous autoxidation of the ascorbate monoanion and/or catalytic oxidation of the ascorbate by the chromium(III) product. The oxidant in both cases would be dissolved oxygen. Another possibility was the reoxidation of any chromium(V) intermediate. These reactions however had no direct bearing on the rate-determining step.

It is possible that oxygen is involved in formation of some intermediate which is non-reactive or reacts quite slowly. This trimolecular system would cause a depletion in the concentration of the reactive species available for the k_f pathway. A lowering of the concentration of reactive molecules would therefore result in a slower rate of reaction. This revised mechanism is shown in Scheme 3. When the oxygen concen-

$$HA^- + [HCrO_4]^- \overset{O_2, K_O}{\rightleftharpoons} I_O \text{ (unreactive or reacts slower)}$$
$$k_f \downarrow \quad k_r$$
$$I \longrightarrow \text{products}$$

Scheme 3

tration is high as under aerobic conditions, $[I_O]$ is large since more reactants are utilized in this pathway. Consequently, k_f decreases as less ascorbate and chromium(VI) are available for reaction. The k_r term can however be excluded for reasons explained earlier. Having removed oxygen, $[I_O]$ is insignificant and most, if not all the reactants, are utilized in the major kinetic reaction, resulting in larger values for k_f.

A ternary metal–ascorbate–oxygen adduct was earlier proposed as a possible intermediate to explain the catalytic oxidation of L-ascorbic acid by metal ions, with the simultaneous reduction of dioxygen.[53] Here, the loosely co-ordinated oxygen is reduced when an electron is transferred from a 2p orbital of ascorbate to a t_{2g} non-bonding, or an e_g antibonding, metal orbital. This is followed by transfer of the electron to an antibonding π_y^* 2p or π_z^* 2p orbital of the oxygen molecule. It was shown *via* X-ray analysis that an olefinic type of metal–oxygen bond exists, demonstrating that the bonded O_2 is not of the peroxo type.[11]

The nature of any adduct involving dissolved oxygen might help explain the role of oxygen in the overall mechanism. Although $[O_2] < [Cr]_T$ in this study, it was noted earlier that only 0.50 mol of oxygen was required for the formation of a stable bis(cysteinato)cobalt(III) complex from cobalt(II).[53]

The absence of a double reciprocal plot involving oxygen concentration is likely to rule against reoxidation or oxygenation of intermediate oxidation states of chromium, namely chromium(V) species.[54] A previous publication[4] has reported the rapid reduction of dissolved oxygen in solution by ascorbate radicals.

These and other points are being thoroughly investigated in

order properly to elucidate the role of dissolved oxygen in this reaction, as the overall mechanism now seems far more complex than had been originally assumed.

Acknowledgements

Funding for this work was provided by the Department of Chemistry, and a Postgraduate Award by the Board for Graduate Studies, University of the West Indies (to D. A. D.) is gratefully acknowledged.

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Received 13*th May* 1993; *Paper* 3/02724D